

REMARKS

Claims 1-48 are active in the present application. Claims 6, 8, 12-13, 15, 17-18 and 22 have been amended to remove multiple dependencies. Claims 23-48 are new claims. Support for the new claims is found in the original claims. The specification is amended to include a substitute Sequence Listing and to change the Sequence Identifiers (SEQ ID NO:) to correspond to the substitute Sequence Listing.

Applicants submit that the sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the foregoing amendments. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518



22850

(703) 413-3000
NFO/DJP/smi

H:\213930US-pr.wpd

213930US-0PCT

Marked-Up Copy

Serial No:

09/926,163

Amendment Filed on:

12-21-01**IN THE SPECIFICATION**

Please amend the specification as shown in the attached marked-up copy to read as follows:

Page 4, beginning at line 22 and continuing to page 5, line 2, delete the paragraph and replace it with the following paragraph:

(5) An SLDH which is the following protein (a) or (b):

(a) a protein consisting of an amino acid sequence depicted in Sequence Listing SEQ ID NO:[1]2

(b) a protein consisting of the same amino acid sequence as

(a) above, except that one to several amino acids are deleted, substituted, inserted, added or modified, which catalyzes a reaction converting D-sorbitol to L-sorbose.

Page 5, lines 5-10, delete the paragraph and replace it with the following paragraph:

(7) The DNA of the above-mentioned (6), which is (a) or (b) of the following:

(a) a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:[2]1

(b) a DNA capable of hybridizing to the base sequence of the above-mentioned (a) under stringent conditions.

Page 5, lines 13-16, delete the paragraph and replace it with the following paragraph:

(9) A gene encoding a protein having an SLDH activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:[2]1 and a partial DNA thereof.

Page 5, beginning at line 20 and continuing to page 6, line 1, delete the paragraph and replace it with the following paragraph:

(11) A promoter gene comprising the DNA of the following (a) or (b)

(a) a DNA having a base sequence of base numbers 1 - 536 of the base sequence depicted in Sequence Listing SEQ ID NO:[2]1

(b) a DNA having a base sequence of the above-mentioned ~(a) wherein one to several bases is (are) deleted, substituted, inserted, added or modified, which DNA shows a promoter activity at least in one microorganism.

Page 8, beginning at line 21 and continuing to page 9, line 21, delete the paragraph and replace it with the following paragraph:

The SLDH of the present invention is not particularly limited as regards the derivation as long as it shows the above-mentioned characteristics. It may be derived from a naturally occurring organism, a spontaneous or artificial mutant, or a transformant which is obtained by introducing a heterologous (i.e. foreign) SLDH gene. Preferably, SLDH derived from acetic acid bacteria, particularly bacteria belonging to the genus *Gluconobacter*, more preferably *Gluconobacter oxydans*, particularly the strain *Gluconobacter oxydans* 6624 (FERM BP-4415; International Patent Publication No. W095/23220) are exemplified. In another preferable mode, the SLDH of the present invention is an SLDH derived from the same gene as is the SLDH derived from the strain *G. oxydans* 6624 in its molecular evolution. As used herein, by the "derived from the same gene ... in its molecular evolution" is meant an SLDH reasonably concluded to have evolved from the same gene as has an

SLDH derived from strain *G. oxydans* 6624 in its molecular evolution, as a result of the analyses of DNA sequence, physiological role and the like, and their DNA sequences show high homology. These SLDHs preferably have not less than 60%, most preferably not less than 80%, homology in the DNA sequence with an SLDH derived from the strain *G. oxydans* 6624. These genes can be cloned based on the DNA sequence depicted in Sequence Listing SEQ ID NO:[2]1 and using a suitable primer according to the PCR method or using a suitable probe according to the hybridization method, as detailed later.

Page 9, beginning at line 22 and continuing to page 10, line 8, delete the paragraph and replace it with the following paragraph:

In a more preferable mode, the SLDH of the present invention is a protein having an amino acid sequence depicted in Sequence Listing SEQ ID NO:[1]2 or a protein having an amino acid sequence having the amino acid sequence comprising one to several amino acids deleted, substituted, inserted, added or modified, as long as the SLDH activity is not impaired.

Page 11, lines 11-16, delete the paragraph and replace it with the following paragraph:

Production of the SLDH of the present invention by chemical synthesis includes the steps of, for example, synthesizing, based on the amino acid sequence depicted in Sequence Listing SEQ ID NO:[1]2, the entirety or a part of each sequence using peptide synthesizer, and renaturating the obtained polypeptide under suitable renaturation conditions.

Page 17, lines 9-26, delete the paragraph and replace it with the following paragraph:
A DNA encoding the SLDH of the present invention preferably encodes an amino acid sequence depicted in v. Sequence Listing SEQ ID NO:[1]2, or an amino acid sequence wherein, in the above-mentioned amino acid sequence, 1 to several amino acids are deleted,

substituted, inserted or added (provided that a protein consisting of the mutated amino acid sequence can catalyze the reaction to convert D-sorbitol to L-sorbose). More preferably, a DNA encoding the SLDH of the present invention is a DNA substantially consisting of a base sequence having a base number 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:[2]1. As used herein, by the "DNA substantially consisting of" is meant a DNA consisting of this specific base sequence and a DNA consisting of a base sequence capable of hybridizing to the DNA consisting of this specific base sequence under stringent conditions, and encoding a protein having similar physicochemical properties as the protein encoded by the DNA consisting of this specific base sequence.

Page 18, lines 6-14, delete the paragraph and replace it with the following paragraph:

The DNA of the present invention may be a DNA obtained from a genomic DNA as mentioned above, or a cDNA obtained from mRNA, or DNA chemically synthesized based on a base sequence having a base number 537 - 1991 from the base sequence depicted in Sequence Listing SEQ ID NO:[2]1.

Page 18, lines 15-19, delete the paragraph and replace it with the following paragraph:

The DNA of the present invention may be a DNA obtained from a genomic DNA as mentioned above, or a cDNA obtained from mRNA, or DNA chemically synthesized based on a base sequence having a base number 537 - 1991 from the base sequence depicted in Sequence Listing SEQ ID NO[2]1.

Page 18, beginning at line 20 and continuing to page 19, line 8, delete the paragraph and replace it with the following paragraph:

The DNA encoding SLDH, which is obtained from a genomic DNA with the SLDH activity as an index as mentioned above, contains a promoter gene sequence in the 5' upstream region. This promoter gene preferably has a base sequence having a base number 1 - 536 from the base sequence depicted in Sequence Listing SEQ ID NO:[2]1, or said base sequence wherein one to several amino acids are deleted, substituted, inserted, added or modified, which is a DNA having a promoter activity in at least one microorganism. As the "microorganism" here, there are preferably exemplified prokaryotes such as bacteria (e.g., *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas*, *Gluconobacter*, *Pseudogluconobacter*, *Acetobacter* and the like) and actinomycetes, and certain eucaryotes such as yeast and the like.

Page 35, lines 16-26, delete the paragraph and replace it with the following paragraph:

Using plasmids pUCP19-HC, pUC18-S1, pUC18-ES and pUC18E1 as templates and using universal primer and reverse primer (New England Labs.), which were M13 sequencing primers, first sequencing was performed. The sample was fluorescent labeled with BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and analyzed with ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The following 11 kinds of primers were synthesized and using pUCP19-HC as a template sequencing was performed, whereby the base sequence of about 4 kb Hind III/Hind III fragment was determined (Sequence Listing SEQ ID NO:[2]1).

Page 48, after the last line beginning on a new page, please replace the original Sequence Listing with the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as follows.

-- 6. (Amended) A DNA encoding the sorbitol dehydrogenase [of any of claims 1 to 5] as claimed in claim 1.

8. (Amended) The DNA of claim 6 [or 7], which is derived from bacteria belonging to the genus *Gluconobacter*.

12. (Amended) A recombinant vector comprising a DNA [of any of claims 6 to 9] as claimed in claim 6.

13. (Amended) An expression vector comprising a DNA [of any of claims 6 to 9] as claimed in claim 6.

15. (Amended) A transformant obtained by transforming a host cell with an expression vector of claim 13 [or 14].

17. (Amended) The transformant of claim 15 [or 16], which is capable of converting D-sorbitol to 2-keto-L-gluconic acid.

18. (Amended) A method for producing a protein having a sorbitol dehydrogenase activity, which method comprises culturing a host cell transformed with an expression vector of claim 13 in a medium and harvesting the sorbitol dehydrogenase [of any of claims 1 to 5] having the following properties

(a) action: catalyzes the reaction converting D-sorbitol to L-sorbose

(b) molecular weight: about 54 kDa

(c) coenzyme: NAD(P)⁺ dependent

(d) substrate specificity: specifically oxidizes sorbitol, mannitol and arabinol, but does not act on xylitol, ribitol, inositol or glycerol, or [the protein of claim 10] a protein derived

from the genus *Gluconobacter*, which is encoded by a gene encoding a protein having a sorbitol dehydrogenase activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:2 and a partial DNA thereof, and which has sorbitol dehydrogenase activity,
from the obtained culture.

22. (Amended) A method for producing L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof, which method comprises converting 2-keto-L-gluconic acid obtained by the method of claim 20 [or 21] to L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof.

Claims 23-48 (New).--